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A CLINICAL AND LABORATORY INVESTIGATION  
on  
the co-relations of the ductless glands and  
vegetative nervous system with special reference  
to this co-relation in Graves' disease.

Thesis for the degree of M.D.

by

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## INTRODUCTION.

In recent years much work has been done, especially in France and Germany, concerning diseases of the ductless glands. Considerable light has thus been shed upon the significance which these glands have in the metabolic processes of the body. There is still however much to learn concerning the co-relations of these glands both in physiological and pathological states. Since more widely spread attention has been drawn to their importance, in comparatively recent years many theories regarding their respective functions have been propounded, and it has been suggested that failure or hyperactivity of one or other of these glands is the pathological basis of many diseases or clinical syndromes whose origins are still obscure. Thus changes in the function of these ductless glands produce alterations in the blood picture, which in many cases has an important bearing in regard to the diagnosis, prognosis and treatment of many disease conditions.

In the course of a discussion at the British Medical Association meeting in July, 1910, Kocher stated that in doubtful cases of Graves' disease he was unwilling to accept the affirmative diagnosis if the/

the blood test were not positive. The weight of such an authoritative opinion is such that one is forced to consider if sufficient clinical study is made of the blood conditions in this and other diseases supposed to be due to changes in the ductless glands. Consequently during the past six months I have been engaged in investigating experimentally the effects which glandular extracts produce on the relations of the blood cells, with the view of finding if possible some answer. I am now enabled to place before you detailed observations which appear to show that the proportional distribution of the various blood cells is clinically of the greatest importance in the diagnosis, prognosis and treatment of certain pathological conditions of various of the ductless glands. I have been able to make these observations from numerous experiments upon rabbits with atropine, pilocarpine and the extracts of thyroid, suprarenal and pituitary. Although conducted upon animals they substantiate the observations made by me in cases of disease of the ductless glands admitted to the wards of Doctors Eason and Matthew in Leith Hospital.

These results of animal experimentation also bear out the blood conditions found in diseases of the ductless glands by such observers as Kocher and Kostlivý.

TECHNIQUE OF EXPERIMENTAL WORK.

To obviate as far as possible the many experimental errors which frequently occur, every precaution was taken regarding the weight and feeding of the animals, and much care was given to the method of collecting the blood and counting and staining. The animals were kept in the same room and got the same kinds of food, the amount being roughly weighed so that they should receive the necessary quantity to keep them as far as possible at a steady weight. Any animal that showed a large increase or decrease in weight was not used in the experiments. The animals were fed in the evening and any food that remained over in the morning was removed. No food was given during the day. The blood was taken from a vein of the ear, and every care was taken to avoid areas of skin that had become oedematous after preceding punctures. All unnecessary friction which might in any way alter the results of the blood count was avoided. The normal blood counts were made as nearly as possible at the same time of day and at the same time after food so that digestion leucocytosis or diurnal variations in the blood could/

could be practically discounted.

The counts were made with the Thoma-Zeiss haemocytometer and the haemoglobin estimated in percentage of human by Haldane's haemoglobinometer.

As the rabbit's blood contains pseudo-eosinophils, which with some methods of staining resemble the true eosinophils, several stains were tried to enable me to differentiate these cells with absolute certainty. I arrived at the conclusion that the most reliable method was to stain with eosin for two minutes and methylene blue for four minutes. By this method the granules of the pseudo-eosinophils either remain unstained or take on a dark mauve color with both dyes, while the true eosinophils stain dark or light red with the eosin.

After many normal counts had been done over a considerable period, I found that there was very great variation in the peripheral blood count of different rabbits. On the other hand I found that the blood count remained fairly constant in the same rabbit under regulated diet and exercise. Moreover the variation occurring from hour to hour during the day was extremely small (Vide Table I.) Counts taken at various times of the day showed very little/



little change for the reason that the digestive processes are practically constantly and fully active. The habit of the rabbit is to keep the stomach loaded with food and consequently there are no decided changes approaching in degree the digestion leucocytosis of man. Experiment on Table I shows that even if only fed during the night, the blood count manifests very little change during the day.

It may be here noted that the temperature of rabbits rises slightly as the day progresses.

Before being used for the following experimental observations all the animals were kept under the same conditions with carefully regulated diet till the weight and peripheral blood count remained at a fairly constant standard.

#### EXPERIMENTAL RESULTS.

##### A. Atropine.

##### Technique of Experiments with Atropine:-

In all experiments the weight of the animal was ascertained and the normal count taken during the morning between the hours of 10-30 and 11-30. Thereafter, and as soon as it was possible to do so, the injection was given. Counts were then taken at various recorded periods to ascertain the effects produced/

produced on the peripheral count.

As Binz found that .02 gms. of atropine caused paralysis of the vagus in a rabbit of 1800 gms., a dose smaller than this was resolved upon. Several experiments were done with .012 gms. atropine sulphate per kilo. of body weight. The atropine was dissolved in 1 cc. of .85% normal saline and injected subcutaneously into the flank of the animal.

#### General Effects :-

The rabbit was dull and listless. Dilatation of the pupils occurred in half an hour and lasted for five or six hours. The temperature (taken per rectum) was raised in all experiments -  $1 - 1\frac{1}{2}^{\circ}$  F. in half an hour and continued to rise for six hours, then gradually fell to normal. The maximum rise amounted to  $2 - 2.5^{\circ}$  F.

#### Results on peripheral Blood count :- (Vide Tables - II. and III.)

- I. Gradual increase in number of erythrocytes in all experiments the maximum being reached in five hours after the injection and thereafter regaining the normal in twenty-four hours. In two experiments nearly 1,000,000 increase was obtained.
2. The haemoglobin at first was reduced then gradually/

gradually rose but at a later period than the erythrocyte rise. It was subnormal in twenty-four hours.

3. The leucocytes showed in all experiments a well marked leucopenia, noticeable in half an hour and reaching the maximum in two hours. The fall affected chiefly the polynuclear and large mononuclear elements both of which showed a percentage as well as an absolute decrease. The neutrophils and eosinophils also showed an absolute decrease but the percentages on the other hand were either unchanged or slightly increased. Three hours later a gradual increase was noticed resolving itself into a hyperleucocytosis. This was due to a large relative and absolute increase of the neutrophil cells (Vide table II. Expt. 3.) The small lymphocytes showed either a continued relative and absolute fall or but a slight increase in absolute numbers with the higher total count. In the latter circumstances however the percentage was always reduced. The eosinophils showed in one to two hours a relative and absolute increase followed five hours later by a return to normal or slight decrease. The large mononuclears followed very closely the rise and fall of the neutrophils, showing at first a relative and absolute decrease and later a rise both relative and absolute.

After/



After a second and third injection the peripheral blood count showed in all respects similar though less pronounced alterations in the distribution of the blood cells.

#### E. Pilocarpine.

The technique was the same as that followed for the atropine experiments.

Subcutaneous injections of .012 gms. per kilo. of nitrate of pilocarpine were used in all experiments.

##### General Effects :-

In all cases there was observed within half an hour marked shivering, excitability and contraction of the pupils. Later symptoms were copious salivation, <sup>and</sup> excessive secretion from nose and eyes. Large watery motions, containing mucous, were passed on the slightest excitement or movement.

Two hours later considerable abatement of these symptoms had taken place but the excitement was still fairly well marked. Five hours after the injection the acute symptoms had for the most part passed off - languidity and listlessness now being the chief features. Repeated injections seemed to produce a toleration as the symptoms became less and less marked/

marked with each injection.

In all experiments reduction of temperature was noticed - 1 - 2<sup>o</sup> F. being obtained in half an hour with still further fall in two hours - followed by a gradual rise to normal in five hours. Repeated injections produced the same drop in temperature but the maximum fall occurred earlier, namely one hour after the injection.

Effects on peripheral Blood count :- (Vide - Tables  
IV - IX.)

1. In five of the six experiments undertaken with pilocarpine the erythrocytes show an increase varying from 200000 - 700000, noticeable in half an hour, continuing for two hours, and followed by a gradual return to normal. In two experiments the normal was reached in five hours but in two others this was not attained till twenty-two to twenty-four hours after the injection. During the phase of erythrocytosis half an hour after its onset there appear nucleated red corpuscles. These are to be found during a considerable period of time after the count has fallen to normal - in one case forty-eight hours ( Vide - Table V. Expt. 2.)

In two cases in which repeated injections were given/

given the erythrocyte count was increased by over 1,000,000 - each injection being followed by a further increase in the number of red corpuscles and by the entrance of nucleated reds into the peripheral blood. (Vide - tables VIII and IX. Expt. 5 & 6)

2. The percentage amount of haemoglobin corresponded closely in its rise and fall with the changes in the numbers of erythrocytes.

3. In four of the six experiments in which the blood count was taken half an hour after the injection hyperleucocytosis was well marked. This was followed after one hour by a slight leucopenia which lasted for 2 - 4 hours and gradually returned to normal in 22 - 24 hours. Of the other two experiments one showed a hyperleucocytosis in one hour, the other showed no primary hyperleucocytosis. The primary hyperleucocytosis is due to a relative and absolute increase of the small lymphocytes and this is accompanied by a relative and absolute decrease of the neutrophils, eosinophils and large mononuclears. Following upon these changes there is a gradual relative and absolute increase of each of these varieties of cells until the normal equilibrium is attained in from twenty-four to forty-eight hours.

In three experiments in which repeated injections were/

were given there were produced, although to a less marked degree, the same changes in the total leucocyte count. In these experiments however, the neutrophil, eosinophil and large mononuclear cells show a cumulative increase, while the small lymphocytes show a progressive decrease.

### C. Atropine + Pilocarpine.

As atropine - a vagal depressant - produces in the cellular distribution of the peripheral blood contrary changes to those which pilocarpine produces, I therefore conducted two experiments to enable me to determine the effects which the combined actions of these substances might produce.

In these experiments atropine was given half an hour before the pilocarpine injection as the effect of atropine is more slowly produced. Both substances were given by subcutaneous injection in the same doses as before - viz :- .012 gms. per kilo of body weight.  
General Effects :-

It was found that atropine more than counter-balanced the effect produced by the pilocarpine. There was no noticeable excessive secretion from the nose, mouth or eyes and there was no excessive diarrhoea. Only three or four soft motions were passed/

passed half an hour after the combined injection.

The pupils were only slightly dilated but the dilatation remained for the same length of time as in experiments with atropine alone.

Effects on the peripheral blood count :- (Vide -  
Table X. Expts. 1 and 2.)

1. The erythrocytes and haemoglobin showed in both experiments an increase similar to that obtained in experiments with atropine or pilocarpine alone.
2. In one of the two experiments in which a blood count was made half an hour after the first injection the leucocytes showed a decrease with reduction in the number of neutrophils, eosinophils and large mononuclears - the decrease obviously being due to the atropine. In both experiments when the atropine and pilocarpine had presumably become effective a rapidly increasing and much larger hyperleucocytosis than had occurred in previous experiments with atropine or pilocarpine alone, was the result. This hyperleucocytosis was due to increase of the neutrophil, eosinophil and large mononuclear elements, the small lymphocytes on the other hand showing a marked relative and absolute diminution.

#### D. Adrenalin.

The/

The technique was the same as that followed for experiments with atropine and pilocarpine. Two preparations of the active principle of the suprarenal gland were used, (Adrenalin. Parker Davis & Co.; and Vasoconstrictine. Duncan Flockhart & Co.) and it was found that they gave the same results. Varying doses of .00025 gms. - .003 gms. per kilo. of body-weight were dissolved in 1 cc. of slightly acidulated normal saline and injected subcutaneously. In four of the eight animal experiments in which Löwi's reaction was tried there was a positive result.\*

#### General Effects :-

In seven experiments the following symptoms were observed :- languor, coldness of the surface with constriction of the superficial vessels, increased flow of urine and lowering of the temperature, the amount of the fall depending upon the dose of the injection. This fall of temperature was first noticeable two hours after the injection, and there was/

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\* Löwi reaction - A drop of adrenalin solution is instilled into the eye followed five minutes later by another drop. If the reaction be positive there is produced dilatation of the pupil. This reaction which may last quarter to half an hour takes place within one hour after the instillations. According to Löwi it denotes hypertonicity of the sympathetic system.



was a progressive decrease for six hours when the maximum fall was attained. Thereafter there was a gradual rise to normal. The maximum fall observed after a non-lethal dose was 4 - 5 <sup>0</sup>F. and in a fatal case 7.5 <sup>0</sup>F. before death.

Effects on the peripheral blood count :-

(Vide - tables XI. - XV.)

- 1- There was noted a large increase in the number of erythrocytes in two hours after the injection. This increase continued for twenty-four hours or longer. With the erythrocytic increase there were numerous nucleated red corpuscles. These increased in number during the twenty-four hours following<sup>upon</sup> the injection and then decreased. In one experiment (Vide - table XIII. Expt. 5) the blood film taken forty-eight hours after the injection showed in terms of white cells 40 per thousand of nucleated reds. (Normally the rabbit's blood contains 4 - 6 per thousand nucleated reds in terms of white cells.) The erythrocyte increase was proportional to the dose of the injection.
2. The haemoglobin follows closely the rise and fall in the number of erythrocytes but at a somewhat later period.
3. In four of the seven experiments in which a single injection/

injection was given there was at first a slight leucopenia. The remaining three showed a slight increase. This was followed in all seven experiments by marked hyperleucocytosis enduring for a period of from twenty-four to forty-eight hours. This hyperleucocytosis was found to be due to progressive increase both relative and absolute of the neutrophil and large mononuclear cells. The eosinophils on the other hand in all seven experiments showed a marked relative and absolute decrease, so much so, that in two of the experiments no eosinophils could be found. Twenty-four to twenty-nine hours later they increased again to normal and in three of the experiments they showed an absolute and relative increase. In six of the seven experiments lymphocytes showed a relative and absolute decrease although in one experiment they increased slightly in absolute number.

(Vide - Table XIII. Expt. 5.)

A second injection given twenty-four hours after the first produced a still further polymorph leucocytosis and there was again a reduction in the number of eosinophils and a percentage reduction of lymphocytes. This neutrophil reaction was proportional to the dose of the injection.

In /

In two experiments done on the human subject (one in a chlorotic female of 21 - the other in an elderly woman with severe secondary anaemia) in which a very small dose of adrenalin was injected, the results were the same (Vide - Table XIV. Expt. 8 & 9.) In both cases the erythrocytes and haemoglobin were increased. There was also a slight hyperleucocytosis which in the second case lasted for seventy-two hours. Both cases showed considerable increase of the neutrophil cells with diminution of the eosinophils.

In one animal experiment I gave on forty-four consecutive days injections of a small dose (.00025 gms. per kilo.) of adrenalin. There was little change shown in the number of the erythrocytes or in the haemoglobin percentage. The leucocyte changes however were very interesting. Five days after the injections were started there was a diminished total count with marked absolute and relative diminution of neutrophils and eosinophils. The lymphocytes showed a normal absolute but increased relative count. Twenty-three days after the beginning of the experiment there was still marked absolute and relative diminution along with relative and absolute diminution of large mononuclears. Changes then/

then occurred in the condition of the blood and in the condition of the animal which seem of great importance. From the twenty-fourth day onwards till the death of the animal on the forty-fourth day there was a progressive increase both relative and absolute in the number of the neutrophil and large mononuclear cells, with progressive diminution of the lymphocytes and continued diminution of the eosinophils. This neutrophil increase was accompanied by a gradual onset of nervous symptoms which developed into extreme cerebral and cerebellar irritation before a fatal ending occurred. (Vide - table XV. Expt. 10, also description postea with P.M. report).

On the fifth day after the injections were started Löwi's reaction was positive. This was followed in thirteen days by a negative result which lasted for several days. It again became positive two days before the death of the animal.

Results of P.M. in three fatal cases :-

Rabbit A. - died three hours after a single injection of adrenalin (.0053 gms. = .003 gms. per kilo.)

Rabbit. B. - died three hours after a second injection of adrenalin (.0038 gms. = .002 gm. per kilo.)

Rabbit C. - died after daily injections of .00025 gm per kilo during a period of forty-four days.

Rabbit/

Rabbit C. showed very striking antemortem symptoms which I think were due to the effect of the adrenalin and seem worth recording. A week before death the rabbit became nervous and trembling, these symptoms gradually increased in severity, and two days before death they became very acute. At this time twitching of the mouth and eyelids was noticed. In the evening the head also showed jerking movements and at times the animal drew up its head till retraction was well marked. Next morning there were twitching and shaking of the front legs, as well as grinding of the teeth. These symptoms passed into what resembled convulsions. The rabbit gradually rose up into the sitting position then into a begging position on the hind legs and finally it fell over always backwards. This was followed by a three or four minutes' interval of quietness. This cycle of events was passed through every five or six minutes. During the convulsions the pupils contracted. The animal passed into a semi-paralytic condition and died next day.

The findings in Rabbits A. & B. were :- The stomach was enormously dilated and fairly full with food. The upper part of the small intestine was collapsed and empty, the lower part empty and distended with gas. There/

There were also noticed paralytic distention of the bladder, nutmeg liver and free fluid in the pleural and pericardial spaces. No haemorrhages were to be seen. Rabbit C. showed neither brain lesion nor any evidence of haemorrhage. Although the P.M. was performed five or six hours after death there was noticed great fluidity of the blood, not a vestige of clotting being seen.

Sections of the bone marrow were made in cases A. and C. and smears in all three cases. These showed identical and decided changes viz:- intense erythroblastic and leucoblastic changes most marked in Rabbit A. On naked eye examination the marrow was found to be intensely red while microscopically this change was found spreading in from the periphery and encroaching on the fatty marrow. Also scattered throughout the fatty marrow were areas showing intense erythroblastic change. The normoblasts and megablasts were enormously increased as also were the neutrophilic and eosinophilic myelocytes. Leucoblasts were also abundant. The suprarenals from Rabbits A. & C. showed loosening of the structure and cloudy swelling with increased cellular granulation.

#### E. Thyroid.

Liquor/



Liquor thyroidei (B.P.) was used for all experiments. This was given in doses of .29cc. - .59cc. subcutaneously and .118 and .237 cc. intravenously.

#### General Effects :-

There were no symptoms or changes of temperature to note in any of the experiments.

Effects on the peripheral blood count :- (Vide -  
Tables XV - XVII.)

1. In only one experiment was there a slight increase in the number of erythrocytes twenty-four hours after the injection and in two experiments numbers of nucleated reds were seen. The remaining four experiments showed no change either in the erythrocytes or haemoglobin.
2. The leucocytes showed a slight increase within the first five hours, falling in twenty-four hours to normal or even subnormal and remaining thus for two or three days. Thereafter the counts showed normal numbers. At first there was marked relative and absolute increase in the neutrophil cells with slight relative and absolute diminution of lymphocytes. This was noticed in one hour after intravenous injection and in two hours after subcutaneous injection. The rise continued in all four experiments for five hours/

hours after the injection. In twenty-four hours this was followed by a gradual diminution in the number of neutrophils both relative and absolute with increase of lymphocytes which lasted for three days and then returned to normal. The eosinophils showed a continuous diminution for several days before returning to normal. The large mononuclear cells fell in number during the first few hours and then increased with the neutrophils or at a slightly later period.

In one animal which died ninety-seven hours after an injection there was a marked secondary rise of neutrophils and large mononuclears with renewed decrease in eosinophils and lymphocytes. (Vide - table XVI. Expt. 2.) This secondary rise began in forty-eight hours and continued to increase till the animal's death in ninety-seven hours. The bone marrow showed the same leucoblastic changes as occurred with adrenalin although milder in type. A very slight erythroblastic change was also seen.

In an experiment in which daily injections of a small dose (.118cc.) of liquor thyroidei were administered for twenty-three days there was produced no change in the erythrocytes or haemoglobin after ten days but after twenty-three days there was marked diminution of both. The leucocytes showed a progressive increase in total number with absolute and relative progressive/

progressive increase of neutrophils and large mononuclears and with continued marked diminution of eosinophils. The lymphocytes showed no absolute change but were diminished in percentage. The Löwi reaction which was done twenty-two days after the injections were begun gave a positive result. The animal showed very slight exophthalmos but no other symptoms. (Vide - table XV. Expt. 5.)

#### F. Pituitary.

Two experiments were conducted with Borroughs Wellcome and Co's "Vaparoïe" pituitary. It was given intravenously in doses of .5 cc. and .75 cc.

General effects :-

In both cases languidity was the only symptom noticed. The temperature was reduced by 5 °F. in one hour in both experiments and then rose gradually to normal. It was interesting to notice that in view of the discussion between Dale and Schäfer concerning the sympathetic action of pituitary, Löwi's reaction was positive in half an hour after the injection.

Effects on the peripheral blood count :- (Vide - table XVIII.)

1. In two hours after the injections an increase in/

in the number of erythrocytes was noted. This increase which was accompanied by numerous nucleated corpuscles was maintained for five hours and then there was a return to the normal numbers of the erythrocytes.

2. The haemoglobin varied in accordance with the erythrocytic count.

3. The leucocytes showed a primary leucopenia of one hour's duration with the larger dose, followed by a hyperleucocytosis which lasted for forty-eight hours. With the smaller dose there was a hyperleucocytosis which began in one hour and lasted for ninety-six hours, but no primary leucopenia.

The two cases showed a progressive increase both relative and absolute of the neutrophils and large mononuclears. This increase lasted for twenty-four to seventy-two hours after which there was a return to the normal. The eosinophils showed a marked relative and absolute decrease which began in half an hour and lasted for seventy-two hours before returning to normal. With the rise of neutrophils the lymphocytes decreased both relatively and absolutely and then in twenty-four hours with the neutrophil fall showed a hyperlymphocytosis before falling to a final normal again.

Bertelli/

Bertelli, Falta and Schweeger, who in a recent paper have described similar experiments upon dogs, have put forward the theory that changes in the distribution of the cellular elements of the blood are controlled by a nerve influence, and point out that although the chemotactic theory is not dead, yet it cannot explain the changes produced by certain substances e.g. atropine and pilocarpine and it is still less able to explain the changes produced by the secretions of the ductless glands.

My experiments performed upon rabbits confirm in many points the results obtained by these observers. In their experiments with atropine they found a marked decrease in eosinophils both relative and absolute. This I did not uniformly get in rabbits - some experiments showing an increase, others a decrease. In this respect my observations were inconclusive and the explanation is probably to be found in the fact that rabbits can endure a much larger dose than was given in my series. The dose given was smaller than that which Binz found was necessary to paralyse the vagus.

In my adrenalin experiments I obtained a primary leucopenia which was not observed by Bertelli, Falta and Schweeger in dogs. Lastly with pituitary injections/

injections I observed in rabbits a progressive hyperleucocytosis while these investigators already quoted observed in dogs a primary leucopenia.

There are three possibilities which might account for those marked changes in the peripheral blood stream described in some detail in the preceding pages and in the appended tables. A. It is possible that there may be an alteration in the distribution of the corpuscular elements due to alterations in the calibre of the peripheral vessels with raising or lowering of the blood pressure, or with loss of fluid. B. These changes may be due to the action of chemotaxis. C. They may be controlled by a nerve influence.

In my opinion the first possibility can be quite satisfactorily disproved. After the cutting of the splanchnics or <sup>after</sup> section of the cord there is a fall of blood pressure with leucocyte and erythrocyte diminution. Bertelli, Falta and Schweeger in their experiments with nitrites also point out a primary leucopenia. Now both pilocarpine and thyroid which produce a fall in blood pressure show on the other hand a primary hyperleucocytosis. Again, adrenalin and pituitary each produce an increase of blood pressure, but the first produces a primary leucopenia while/



while the latter produces a hyperleucocytosis. With the primary leucopenia following atropine and adrenalin administration there is an increase in the number of the erythrocytes. These examples suffice to indicate that there is no law governing the relations between blood pressure conditions and the proportional distribution of the various cellular elements of the blood. That changes in the blood pressure or in the total distribution of the blood do not produce in all cases the same alterations in the relationship between neutrophils and lymphocytes can be shown by the observations with nitrites and pilocarpine. Both produce a decrease of blood pressure, but the former according to Bertelli, Falta and Schweeger show an absolute decrease of lymphocytes with increase of neutrophils, while the latter shows an increased primary hyperleucocytosis due to an absolute and relative increase of the lymphocytes with relative and absolute diminution of the neutrophils.

With regard to the second possibility viz., the action of chemotaxis, Bertelli, Falta and Schweeger have dealt fully. They however support their theory not so much by producing evidence in its favour, but by a process of destructive criticism of the theory of chemotaxis and of the experiments which have been published/

published in support of it. They classify atropine pilocarpine and the extracts of thyroid, suprarenal and pituitary under two groups. A. Those that act on the sympathetic system as examples of which adrenalin and atropine are taken. B. Those that act upon the autonomous nervous system under which they classify the extracts of pituitary and pilocarpine.

A point in favour of the nervous theory is, that substances which are known to stimulate the sympathetic system e.g. adrenalin, and those which inhibit the antagonistic system e.g. atropine, should produce similarity in the blood picture, and that pilocarpine which stimulates the antagonistic autonomous system, should produce the opposite effect. This in the light of Langley's dictum that the effects produced by adrenalin upon any tissue are such as follow excitation of the sympathetic nerve which supplied the tissue, seems to point to some nervous influence controlling these blood changes.

Bertelli, Falta and Schweeger state, in favour of their nerve theory, that "as the work which the organism has to do changes rapidly and suddenly, the regulation requires also to take place very rapidly, a fact which from the start indicates that nerve influence/ .

influence plays a great part."

They have nevertheless failed to bring any positive evidence to disprove the chemotactic theory.

#### CONCLUSIONS FROM EXPERIMENTAL RESULTS.

From my experimental results I would point out,

1. The striking similarity in the blood picture produced by adrenalin and pituitary, and also the fact that they both give a positive Löwi reaction. This seems to show that pituitary as well as adrenalin acts upon the sympathetic system.
2. That in experiments in which daily injections of adrenalin and thyroid were given, for a considerable period, there was produced some days afterwards a striking similarity in the blood picture, in the nervous symptoms, in the changes produced in the bone marrow and in regard to the Löwi reaction. Such results have led me to infer that thyroid substance is capable of stimulating the suprarenals to increase the amount of adrenalin passed into the blood.
3. That some time after the daily injections of adrenalin were started, when the blood picture resembled that/

that which is produced by antagonistic vagotonic stimulation, Löwi's test was negative. This conclusion finds a parallel in the results observed by Kostlivý in Graves' disease. During the course of Graves' disease a remission in the symptoms is accompanied by an increase of lymphocytes with negative Löwi. This is apparently due to an antagonistic vagus compensation.

CONCERNING THE IMPORTANCE OF THE BLOOD COUNT  
IN GRAVES DISEASE.

In opposition to Kocher who claims that the essential feature in the blood picture of acute Graves' disease is an absolute diminution of the polymuclear cells, Kostlivý points out that the blood picture shows great variation. He classifies all cases of chronic thyrotoxaemia under three groups.

A. Cases in which the blood shows a lymphocytosis with presence of adrenalinaemia.

B. Cases which show normal or even diminished lymphocytosis with significant or even very marked adrenalinaemia.

C. Cases with hyperlymphocytosis with little or/

or no adrenalinaemia.

He points out that in group B. cases, which show acute clinical symptoms, there is always adrenalin-aemia and never lymphocytosis. The best results follow upon operation when the adrenalinaemia vanishes entirely and the lymphocytes increase to normal. Group C. cases in which there is hyperlymphocytosis with little or no adrenalinaemia, the most serious results follow upon operation if the adrenalinaemia increase rapidly with positive Löwi. He places group A. cases which show lymphocytosis with presence of adrenalinaemia on the border line and points out that very unsatisfactory results are got after operation, three of his eight cases showing increase of adrenalinaemia with ischyperleucocytosis.

My results show the great similarity of effect produced by injection experiments on animals with adrenalin and thyroid. Experiments with single doses of adrenalin show in all cases a progressive increase both relative and absolute of the neutrophil cells with marked decrease in the eosinophils and lymphocytes and with positive Löwi reaction. Daily injection of adrenalin caused in five days an absolute diminution of the neutrophils, eosinophils and large mononuclears, with/

with relative increase of lymphocytes, the absolute number remaining the same, also a negative Löwi which previously had been positive (Vide table XV, expt. 10). Thirty four days later there was produced a progressive increase of neutrophils and large mononuclears with absolute decrease of lymphocytes and eosinophils and positive Löwi accompanied by the onset of acute nervous symptoms, culminating in death. These experiments with adrenalin appear to me to indicate the results of sympathicotonic stimulation upon the blood picture viz. increase of polymorphs and large mononuclears with diminution of lymphocytes and eosinophils, and it is important to note that this is exactly the condition found during exacerbations in cases of Graves' disease. Now, as I have above stated, continuous daily injections produce first an absolute diminution of neutrophils and large mononuclears with lymphocytic increase, and it is very significant that similar changes are produced by stimulation of the antagonistic autonomic nervous system by pilocarpine. In the experiment just described the outburst later of acute nervous symptoms with progressive increase of neutrophils and large mononuclears, shows a preponderance of the sympathetic element which, as the animal died, /



died, the antagonistic vagus seemed unable to compensate. Group A. cases of Kostlivy show a similar outburst with neutrophil increase when the compensatory thyroid hypertrophy had been removed by operation. The absence of a Löwi reaction, when the blood picture showed the effects of the antagonistic vagus activity and the later positive test by the Löwi method with onset of nervous symptoms, lend additional support to the theory that an exacerbation in the course of Graves' disease is due to an increased sympathetic hypertony, while a remission is due to a compensatory vagal stimulation.

Experiments with thyroid (single injection) show at first the effect produced by sympathetic stimulation followed by a prolonged antagonistic vagal effect. Repeated administration however produced a preponderance of the sympathetic element and this view was confirmed by a positive Löwi test. The lymphocytes though relatively diminished showed a very slight absolute increase. The lymphocytic count indicated a state of continual antagonistic vagotony which however was not entirely sufficient to overcome the excessive sympathetic stimulation. There is still further support to be obtained for such a view in the records/

records of an experiment upon a rabbit which died four days after a single dose of thyroid. In this instance there was a renewed progressive increase of neutrophils with decrease of lymphocytes which took place 48 hours after the injection and continued till the animal's death in 97 hours.

In my opinion these results appear to point out,  
 A. That there is an intimate relation between the thyroid and suprarenal secretions and that in some way the thyroid seems to control the secretion of adrenalin. In further support of this opinion I may mention the investigations of Asher and Flack who found that the effect of adrenalin on the blood pressure was rendered greater by stimulating the secretion of the thyroid by means of the laryngeal nerves.

(1)  
 B. That an exacerbation in the course of Graves' disease is brought about by increased sympathetic hypertony due to adrenalinaemia which declares itself by an increase of neutrophils and large mononuclears and a diminution of lymphocytes and eosinophils; and  
 (2) that each remission is due to vagotonic compensation which shews itself in an increase of lymphocytes with diminution of neutrophils and large mononuclears, accompanied by diminution of adrenalinaemia and negative/

negative Löwi reaction, if complete.

THE SIGNIFICANCE OF EOSINOPHILS IN GRAVES DISEASE.

The eosinophils also play an important part in the blood picture of Graves' disease. Michailow, Kocher and others record an increase of eosinophils, while in three cases of my own two showed an absolute diminution and one a normal number.

Experiments with single doses of adrenalin show a diminution followed by an increase. The experiments in which daily injections of adrenalin and thyroid were administered, show a lasting diminution, whereas pilocarpine which is a vagus stimulant produces an increase. These observations suggest the possibility that an exacerbation in the disease produces a diminution of the eosinophils accompanied by an increase of neutrophils, but that when fully compensated, the eosinophils return to normal, or if vagal compensation overbalances the sympathetic hypertony, there is an increase produced. This finds support in the observations of my three clinical cases. In the first two cases in which there were marked/

marked symptoms of hyperthyroidism, one supposed from the diminution of eosinophils that there was incomplete compensation from the antagonistic autonomous system. In the third case, a patient who showed no tremors or tachycardia, although these were formerly present, the blood count showed lymphocytosis with a normal number of eosinophils. In my opinion this blood condition indicated a more complete compensation.

I submit (1) that there is here presented abundant evidence for maintaining that extremely valuable aid for the diagnosis and prognosis of Graves' disease is to be obtained from the complete examination of the blood. (2) That treatment of Graves' disease with thyroid, adrenalin or any other sympathicotonic stimulant would be attended by serious results and (3) That pilocarpine might be given a fair trial in such cases.

THE/

THE BLOOD PICTURE AS AN AID IN OPERATIVE TREATMENT  
OF GRAVES' DISEASE.

As regards operation which is strongly advocated by Kocher and in all cases by Bier, the study of the blood picture is again of much value in helping to a decision for or against operation. In cases in which there is thyroid hypertrophy with lymphocytosis and absolute decrease of the neutrophils, operation would almost certainly be prejudicial as this might result in taking away the compensatory thyroid increase and leaving the primary morbid tissue. This is exemplified by Kostlivy in his C. group of cases in which there were serious postoperative symptoms with increase of adrenalinaemia.

The cases in which operation is likely to be useful are those which show a neutrophil increase, eosinophil decrease and lymphocytes either normal or decreased. Here one can be sure that the sympathetic hypertony is not being compensated. An additional good clinical test is Löwi reaction which if positive shows sympathetic hypertony and which in Graves' disease indicates the presence or absence of adrenalinaemia. Kostlivy says that true hyperthyroidism should be understood as a condition in which increased/

increased concurrent impulses pass from the thyroid to both sympathetic and autonomous systems, with results which may not balance. In consequence either a sympathetic or primary Basedow or a vagal or secondary Basedow develops. In the latter cases operation is followed by exacerbation with renewal of adrenalinaemia plus positive Löwi. This is amply confined by the experiments on animals with continuous daily injections of adrenalin and thyroid, in which there was at first a compensatory change followed by a sympathetic overbalance with outburst of nervous symptoms and renewed positive Löwi.

All my experiments upon animals together with observations from clinical cases point out almost conclusively (1) that every exacerbation is due to a renewed sympathetic hypertony manifesting itself in the blood picture by increase of neutrophils and large mononuclears, by diminution of the eosinophils, by a normal or reduced number of lymphocytes, and by a positive Löwi reaction. (2) They likewise indicate that each remission declares itself by an increase of lymphocytes and eosinophils, by diminution of neutrophils and large mononuclears and by negative Löwi/



Löwi reaction if fully compensated, and (3) that the course of Graves' disease depends on the preponderating influence exercised by the sympathetic or the antagonistic autonomous nerves.

CONCERNING THE CAUSATION OF THE SYMPTOMS AND  
BLOOD PICTURE IN GRAVES' DISEASE.

From the vast amount of literature on the subject the concensus of opinion is that Graves' disease is due to excessive secretion of the thyroid gland acting primarily upon the sympathetic system, the trophic nerve mechanism and the cardio-motor and vaso-motor centres.

But as my experiments have shown that the administration of both thyroid and adrenalin produce eventually the same result viz, adrenalinaemia, then there appears to me to be little doubt but that this adrenalinaemia with its accompanying sympathetic hypertony, typical blood picture, and positive Löwi, reaction is the active cause of Graves' disease and also that it is in some way controlled by the thyroid secretion. Whether this control acts upon the suprarenals/

suprarenals chemically or primarily through the nervous system, I am not prepared to say.

Moreover it has by no means been proved that a primary thyroid excess is in all cases the exciting cause of Graves' disease.

We know on the one hand that the thyroid gland atrophies at about the age of fifty, yet myxoedema does not follow upon its atrophy or upon its surgical removal. On the other hand W. H. Manson found that in fatal cases of Graves' disease in elderly people there was no apparent thyroid left, and that the disease may occur in patients who show no goitre or exophthalmos. Morat was able to produce swelling of the thyroid by exciting the sympathetic nerve and Wiener was able by administering adrenalin to increase the quantity of thyreoglobulin in the thyroid gland.

Now my experimental results with thyroid are very similar to those produced by adrenalin. More especially after repeated thyroid injection there were obtained a blood picture and Löwi reaction similar to that produced by repeated adrenalin injection. This appears to me to indicate that adrenalinaemia was the cause of the blood changes and positive/

positive Löwi reaction in the experiment with repeated thyroid injection, and that this adrenalinaemia had been stimulated by excess of thyroid in the blood.

Moreover, from statistics, Graves' disease in the majority of cases occurs in women especially at the reproductive period of life and it has been noticed experimentally that the suprarenals enlarge during periods of sexual activity and pregnancy.

Is it not possible then that an explanation of the cause of Basedow's disease may be found in primary hyperactivity of the suprarenals, more especially in those cases, which progress unchecked in spite of every medical and surgical measure applied to the goitrous thyroid.

CONCLUSIONS.

- First:- That adrenalinaemia is the exciting cause of the symptoms of Graves' disease and that this adrenalinaemia acts by producing sympathetic hypertony.
- Second:- That a curative effect is produced by a compensatory autonomous stimulation.
- Third:- That, in view of the first conclusion, the administration of any agents which stimulate the sympathetic system should be followed by injurious results.
- Fourth:- That, in view of the second conclusion, vagotonic stimulants e.g. pilocarpine might be employed as a medicinal treatment.
- Fifth:- That the blood should be fully examined and Löwi reaction taken from time to time before any operative procedure is attempted.
- Sixth:- That the pathologic cause of Graves' disease may arise not only from the thyroid but from the suprarenals.
- Seventh:- That owing to the great similarity of the actions of adrenalin and pituitary, and owing to/

to the difficulty of administering adrenalin over a prolonged period without causing injury to the patient (e.g. by hypodermic injection) or without being itself destroyed (oral administration when it is oxidised), pituitary might well be given a trial in diseases in which the former was therapeutically employed and especially in Addison's disease.

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